Dr. Erich Luck's 1972 publication on Sorbic Acid, Vol. II, Biochemistry-Microbiology

Information on Material for **Glutamic Acid** Review fron Dr. Ebert & **Sorbic Acid** Review from Monsanto. 8/8/74 N 36

MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Hearing Clerk, HFC-20

DATE: August 8, 1974

N35

FROM : HFF-335

SUBJECT: Additional material for Scientific Literature Reviews

We attach the following new material for inclusion in the appropriate GRAS substances Scientific Literature Reviews:

1. Material for glutamic acid. Review from Dr. Ebert.
2. Material for Sorbic Acid Review from Monsanto.

Please acknowledge receipt.

Alan T. Spiher, Jr.

Alan T. Spiher, Jr.
Chief, GRAS Review Branch

Attachments
Glutamic acid review
Sorbic acid review

Hearing Clerk

Monsanto

MONSANTO INDUSTRIAL CHEMICALS CO.
800 N. Lindbergh Boulevard
St. Louis, Missouri 63166
Phone: (314) 694-1000

July 26, 1974

George W. Irving, Jr., Ph.D.
Research Associate
Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014

Dear Doctor Irving:

Enclosed are three copies of the translation of Dr. Erich Luck's 1972 publication on Sorbic Acid, Vol. II, Biochemistry-Microbiology, which was not included in the Sorbic Acid Scientific Literature Review, NTIS publication PB-223-864.

We hope this information is useful to the Select Committee on GRAS Substance.

Sincerely,

J. Coleman Weber
Manager, Product Acceptability
Detergent & Fine Chemicals Division

JCW:slv

Enclosures/3

cc: GRAS Review Branch (BF335)
Bureau of Foods, Food and Drug Administration

Translation

SORBIC ACID
Vol. II, Biochemistry-Microbiology
E. Lück, (Behr Verlag. Hamburg), 1972
pp. 13-31

B. Physiological behavior

1. Behavior in plant organisms

At a concentration of 0.1% sorbic acid inhibits the germination of cereals (1) but not the germination of cotton seeds (5). The growth of plant seedlings (cherries, apples, cucumber and wheat) in nutrient solutions is improved by the addition of 0.6 to 5 mg of sorbic acid per liter, but higher concentrations inhibit growth (81). Tests with tops of kitchen onions show that sorbic acid protects against cytogenetic damage by radiation (82)

Pumpkin seedlings, and to a smaller degree their autolysates can utilize sorbic acid; seedlings 4 days old, contain after soaking with 0.1 mol. solution of potassium sorbate considerably more starch and sucrose than controls soaked with water (2). This effect depends on the concentration of sorbic acid not on its amount (3). A relatively substrate-specific saturase is responsible for the conversion of sorbic acid (4).

The above ground portions of higher plants are not damaged by sorbic acid or sorbate solutions, so that the latter were used as agents to fight plant diseases (see Vol. III, p. 140).

2. Behavior in animal and human organisms

a. Acute toxicity: The first tests of sorbic and parasorbic acid for acute toxicity date from 1894. It was found then that parasorbic acid caused in the dog a weak state of intoxication, salivation and vomiting; sorbic acid was harmless in a single dose of 1 g (6). According to more recent data only parasorbic acid is locally irritating but not sorbic acid (78).

In regard to the effect of parasorbic acid it was assumed and is still assumed today that berries of the mountain ash are toxic. But this assumption is contradicted by tests with rats (7). It is also untenable because these berries are valued as food. The tales that mountain ash berries are toxic may be due to some color similarity of the above berries with belladonna and therefore the warning to children to avoid red berries.

Systematic studies on the acute toxicity of sorbic acid were conducted because of its use as conservation additive in food stuff. When fed to rats by an intestinal probe either in the form of a 20%-suspension with a wetting agent in agar solution (sorbic acid) or as a 10% aqueous solution (sodium sorbate) the following average LD_{50} values were obtained expressed in g of s.a. per Kg of body weight.

	Breed A	Breed B
Sorbic acid (s.a.)		10.50 ± 1.96
Sodium sorbate (s.s.) (calculated as s.a.)	4.0	5.94

The rats breed A were fasted 18 hours before the tests, rats breed B were not. The difference in toxicity between s.a. and s.s. may be due to different rates of resorption and the various values for LD_{50} of s.s., to the weight and state of nutrition of the experimental animals (8).

Some further data of acute toxicity of s.a. and derivatives after oral feeding.

		LD_{50} in g/kg	Species	Ref.
s.a.	Sorbinsäure	10,5	Ratte	8
		7,36	Ratte	9
		7,5	Ratte	77
s.s.	Natriumsorbat	4—5,94	Ratte	8
		7,16	Ratte	9
		>8,0	Maus	84
Cu sorbate	Kupfersorbat	2,8	Maus	84
Al sorbate	Aluminiumsorbat	>4,0	Maus	84
Zn sorbate	Zinksorbat	>8,0	Maus	84
Methyl sorbate	Sorbinsäuremethylester	10,0	Ratte	77
Ethyl sorbate	Sorbinsäureäthylester	>8,0	Maus	84
		10,0	Ratte	77
Propyl sorbate	Sorbinsäurepropylester	>8,0	Maus	84
Cyclohexyl sorbate	Sorbinsäurecyclohexylester	>8,0	Maus	84
Dimethylaminoethyl sorbate	Sorbinsäuredimethyl-aminoäthylester	5,6—>8,0	Maus	84
Dimeric sorbic acid monoglyceride	dimeres Sorbinsäure-monoglycerid	5,74	Ratte	69
Sorbic alcohol	Sorbinalkohol	1,0	Ratte	77
Tetrabromosorbic acid	Sorbinsäuretetrabromid	1,5	Maus	83
Sorbohydroxamic acid	Sorboylhydroxamsäure	0,35	Maus	101

LD_{50} of some derivatives of s.a. after intraperitoneal application were:

		LD_{50} in g/kg	Species	Ref.
s.s.	Natriumsorbat	2,8	Maus	84
Copper sorbate	Kupfersorbat	<0,006—0,125	Maus	84
Al sorbate	Aluminiumsorbat	>4,0	Maus	84
Zn sorbate	Zinksorbat	<1,0	Maus	84
Methyl sorbate	Sorbinsäureäthylester	5,6—8,0	Maus	84
Propyl sorbate	Sorbinsäurepropylester	5,6—6,0	Maus	84
Cyclohexyl sorbate	Sorbinsäurecyclohexylester	8,0	Maus	84
Dimethyl amino ethyl sorbate	Sorbinsäuredimethyl-aminoäthylester	1,4—2,8	Maus	84
Sorbo hydroxamic acid	Sorboylhydroxamsäure	0,7	Maus	72
Parasorbic acid (γ-lactone of 2-hexanoic acid, 5-hydroxy-)	Parasorbinsäure	0,75	Maus	118

Intramuscular injections of 35 mg of sorbic acid, sorbyl alcohol or propyl sorbate per kg of body weight did not cause any damage in the mouse (114).

A single dose of 2 g of s.a. per kg of body weight in the rat caused an increase of bile secretion, and a rise of the lipase- and amylase activity of the bile. The content of total proteins, of chymotrypsine activity and the content of cholesterol were lowered; no change was seen in the amount of bilirubin, sodium and potassium (185-87). According to other tests s.a. inhibits amylases only little (105) or not at all (110) and the same is true for pepsin (106).

While β-glucuronidase of the kidney or liver of the mouse in not inhibited in vitro by s.a. in 0.02 M concentration (102), a single injection s.c. of 240 mg of s.a. per kg. caused after 4 days a reduction of the enzyme activity in the kidney and liver, but not in the uterus (103). An intrauterine application of s.a. gave the same result (104).

Sorbic acid inhibits the catalase of beef liver at a pH = 4.5 at a concentration of 0.01 M only weakly (108, 109, 111), and not at all at a pH = 7.0 (108, 110).

b. Subacute toxicity: Feeding tests lasting 28 days with a diet containing 8% of s.a. in various groups of rats (the total s.a. absorption per rat 14.4 or 13.4 g) caused an increase in body weight versus control groups, thus not statistically controlled. The final weight of rats fed with s.a. was not different from a group of rats fed equal amounts of caproic acid. Histological studies and weight measurements made at the end of tests of the liver, spleen, kidneys and intestinal tract show no deviations from normal (11).

Feeding of 40 mg of s.a. per kg of body weight to starving mice over a 2-3 month period results in a small loss of weight as occured in control animals. The resistance of the animals against toxic administrations of carbon tetrachloride wa s considerably increased by s.a. (115).

Feeding diets containing 10% of s.a. for 42 days proved to be harmless (12). Doses of 10 mg of s.a. per kg of feed improved slightly the assimilation of food in most chickens; 1000 mg. of s.a. per kg of feed showed no harmful effect within a 42 day period (67). Rabbits tolerated 3.3-3.4 g of s.a. per day and kg of body weight, without any side effects (14).

Concentrations of 1% or 2% of s.a. in the diet did not influence the growth of rats in tests lasting 80 days and did not cause any histological changes in the internal organs. However, versus control animals a slight enlargement of the liver was seen (14)

Feeding tests lasting 90 days with rats with an 8% concentration of s.a. were harmless. (8% of s.a. in the diet corresponds to a daily consumption of about 5 g. of s.a. per kg. of body weight). The gain in body weight of the animals during the entire testing period showed no difference versus control animals. In the group of animals receiving 8% of s.a. in the diet a slight increase in the weight of the liver occured (calculated in % of body weight). Animals receiving only 4% of s.a. were not different from control animals. No specific histological or pathological changes occured in any group. The weight and structure of the kidney was also unchanged versus the control animals. On the basis of these tests amounts of 4% of s.a. in the diet can be considered certainly harmless, and 8% as probably harmless (8).

Dogs of both sexes received for 90 days a diet containing a maximum of 4% of s.a. calculated to the dry material. No specific changes were seen either in the curve of weight or as seen by the histological studies of liver, kidney, heart, lungs, pancreas, stomach, small intestine, spleen, adrenal glands, thyroid, parathyroid, sex glands, muscles or skin nor in the blood hemoglobin content.

In feeding tests lasting 4 months with potassium sorbate in amounts ranging from 100 and 500 mg per kg. of body weight in male rats no lowering of the blood content of catalase or glutathion was observed. The blood profile was not changed. Under the influence of 500 mg of s.a. per day increase in the body weight occured up to 30% of the corresponding weight of control animals. Animals fed with s.a. were during the testing period more resistant and had a cleaner and shinier fur than the controls (55).

In similarly conducted feeding experiments with male and female albino rats a diet given ad libitum of 10% s.a. was well tolerated. The tests were lengthened to 120 days. The reproduction and growth capacity of the animals remained unchanged. In this test some animals showed in the liver a certain increase of its weight and a stronger formation of fat deposits. These effects were seen only in male animals. Succinatoxidation by liver homogenates was not lowered by administrations of s.a. only in the second generation (10).

In feeding tests lasting 4 months with 1% of s.a. in the diet of rats the content of cholesterol in the blood was not affected. Doses of 10%, however, cause a rise in the blood cholesterol particularly in the amount of bound cholesterol. Also in tests lasting four months with 10% of s.a. in the diet some fat deposits of the internal organs were seen. Next germ content of the feces of the animal rose under the influence of 1 and 5% of s.a. in the diet, but became normal after about 4 months. 10% of s.a. in the diet causes a continuous decline of the germ count. The leucocyte count does not change at all after feeding of 1% and 5% versus control animals, but is lowered after 2-months long administrations of 10% of s.a. Such large doses of s.a. when given over prolonged periods of time cause obviously also a partial inhibition of the cholinesterase activity of the blood (56).

In concentrations of 0.5 to 2% over a period of feeding lasting 3 months both sorbic acid and potassium sorbate cause in the rat a slight decline of the total protein content of the activity of chymotrypsin and amylase of the pancrease secretions. At the same time the extent of biliary secretion which stimulates the activity of lipase and the content of bilirubin and cholesterol in the bile are slightly increased. The amount of sodium and potassium in the bile was not changed. After feeding of 0.25% of s.a. or potassium sorbate the secretion of pancrease, the protein content of the pancreatic juice and the activity of all studied pancreas enzymes was heightened. 2% of s.a. show a contrary affect on the enzymes of pancrease (85-87). According to our studies s.a. is without effect on tryptic activity (91).

Feeding sorbic acid, sorbic alcohol and propyl sorbate in 1 ml amounts as a 1% oily solution for 20 days increases the phagocyte activity of blood leucocytes against staphylococci, and the maximum is reached on the 15 to 20th day. The resistance of the animal against staphylococcal infection was increased about 6-times. Sorbic acid and propyl sorbate were the most effective (114).

Addition of 0.002-0.11% of s.a. in the drinking water of rats over a time period of 20-28 days is without influence on the survival time after irradiation with x-rays over the entire body (700-800 R) (119).

From the presently known studies it should be concluded that even very high doses of s.a. in feeding tests of average length do not cause any damage. When we consider that sorbic acid is normally added to food stuffs in about 0.05 to 0.2% amounts we obtain for the average toxicity a very high range of certainty. The occasionally seen enlargements of the liver may mean a working hypertrophy and in no case should be considered as pathological facts (15).

c. Chronic toxicity: A very thorough study on the chronic tolerance of s.a. was made with about 700 rats and lasted over several generations. 5% of s.a. (daily uptake per rat 0.75 g) in the diet caused a statistically controlled inhibition of growth. Also in the second generation this is assumed to be caused by the increased supply of calories which in a prolonged uptake of 5% of s.a. in the diet already affects the weights. The motor behavior of rats whose diet contains 0.1% to 0.5% of s.a. shows no peculiarities, also few are found in histological studies of the internal organs of the animals whose second generation received 5% of s.a. in the diet. The reproduction capacity of the animal was not disturbed either under the influence of s.a. (15).

In males doses of 0.1%, 0.5% and even more of 5% of s.a. in the diet cause a lengthening of the average life span. Probably the rate of early deaths is reduced versus control. Animals receiving s.a. are more resistant to infections, particularly lung infections which are the immediate cause of death in about 70-80% of all rats.

In females such effects were not observed (15, 16, 89).

Similar results were obtained in tests with alltogether 1900 white mice of both sexes and 400 rats over a period of 18 months. Doses of 40-80 mg of s.a. per kg of body weight resulted in an increased lifespan of ca 8%, observed only in males as in the previous studies (15). In starved animals the lengthening of the life span was also particularly strong which may be related to the caloric metabolism of s.a. As in the average length test the consumption of s.a. improved the ability of the animals to resist toxic gases of CCl_4. Physically troubled mice behaved better than controls after s.a. was introduced.

Prolonged feeding with s.a. did not cause any changes in the macro- and micro structure of the internal organs (54, 115).

No tumor formation was found anyplace in long-lasting feeding experiments neither under the influence of s.a. (54, 68, 79, 115) nor of potassium sorbate (99).

Feeding of 1.2% sa/kg body wt. to rats over a period of 22 weeks was visible by a higher secretion of pancreas and increased content in this hormone of total proteins and potassium, also increased lipase- and amylase activity. Chymotrypsin activity and the content of bilirubin and cholesterol were not affected (85-87).

Parasorbic acid injected subcutaneously twice weekly during 32 weeks caused formation of sarcomas at the injection site only if the latter remained unchanged. This effect seemed to be related to the structure of parasorbic acid as an unsaturated lactone. Similar reactions were observed with other unsaturated lactones lactams and anhydrides of 4 or 5- atom rings. For example penicillin G, but not when aliphatic compounds were used (58, 59, 61, 62). This sarcoma-causing effect is related to a reaction with the SH-groups of cystein (64). Sarcoma formation was also observed with s.a. when an oily solution of 2 mg of the latter was injected subcutaneously at the same place for 65 weeks (68, 98). The sarcomas remained localized on the injection site and were not observed in repeated tests on rats with s.a. of a different origin (99), and likewise after multiple subcutaneous applications lasting 6 months as an oily suspension in mice (74). 0.2% solutions of s.a. have a pH value of 3.3 and due to this acid reaction cause a beginning necrosis in the subcutaneous layer and in the binding tissues. Since s.a. even in oily solutions passes partially in the aqueous phase because of its solubility in water the sarcoma formation of its solutions is caused solely by the damages of tissues that occured in repeated injections of acid solutions at the same body site. (88, 100). Besides, appearance of sarcomas after injection of foodstuff additives should not be considered as possible carcinogenicity as long as the sarcomas remain localized and as long as no other proof of a cancèrogenic effect is present (73, 100).

Potassium sorbate does not cause sarcomas neither after repeated subcutaneous injection (88, 99) nor after feeding (99). Likewise malignatnt changes do not occur after feeding s.a. (68). Consumed s.a. in mice after abdominal injection of 1.2 million cells of ascite tumor had no influence on further development of tumors. These animals which received for 66 days 0.2% of s.a. in the diet had a life span and new growth development similar to control animals (75).

d. <u>Effect on the utilization of other nutritional compounds:</u> Sorbic acid despite its strong unsaturated nature has no effect as metabolic antagonist of linoleic acid. Even 5% of s.a. in the diet had no influence on the concentration of linoleic acid or on symptoms of deficiency in essential fatty acids (8). S.A. behaved obviously in a similar manner as other conjugated unsaturated nutritional fatty acids, about which it is also known, that they are without influence on the metabolism of essential fatty acids.

Rats whose diet contained 10% of s.a. behaved in regard to the cholesterol level in serum similarly as if fed equal amounts of corn-germ oil, rich in higher unsaturated fatty acids (12).

In feeding tests with rats that received 10% of s.a. and lasted 70-90 days the serum cholesterol level had the lowest value compared to animals to whom the fat was added in the form of corn oil, triolein or tripalmitin. The total cholesterol in the liver was also at the lowest level.

Resorption of fats is increased by s.a. But s.a. cannot be considered as a replacement of essential fatty acids. Rats that received a fat-free diet but containing 10% of s.a. had a shaggy fur and suffered diarrhea. They showed symptoms that that could be related to the lack of unsaturated fatty acids (17). When feeding a synthetic fatless diet thus optimal to which coconut oil or corn-germ oil or s.a. were added, s.a. could partially compensate for the deficiency in unsaturated fatty acids; after 15 weeks the rats that received s.a. behaved better than the animals that were fed with coconut fat (18).

Utilization of egg-white did not show any deviation from normal under the influence of 0.1-0.5% of s.a. in the diet even in long-lasting tests (15). When rats with a deficiency of vitamin-B artificially caused were fed for 82 days a diet containing 8% of s.a. neither their food utilization nor life span were affected. Lack of vitamin-B had no unfavorable effect on the behavior of s.a. in the body. The animals gained weight even more than the controls despite the fact that the diet of animals fed with s.a. was made slightly deficient in fat and carbohydrate due to the caloric utilization of s.a. (19, 20). Sorbic acid did not influence the enteric vitamin synthesis (90).

After 14-28 days of feeding a carotene-containing diet with 2% of s.a. the content of vitamin A in the liver but not in the kidneys was lower than in control animals, but not really significantly different; s.a. obviously inhibits the resorption of carotene from paprika puree and increases elimination when it is fed in large amounts (13).

Due to the caloric value of s.a. sorbates of amino acids were proposed for parental feeding (21).

e. <u>Physiological degradation:</u> S.a. is not decomposed in vitro by lipoxydase (112), pepsin (110), trypsin (110) and preparations of diastase (110). N-sorboylglutamic acid, - alanine and -sarcosine are not split by proteases, slowly by pancreas enzymes (lipase-amylase-trypsin- mixture) and rapidly by acylase I (107).

Surviving organs such as dog's liver, give the same degradation product with s.a. as with caproic acid. It was assumed on the basis of these tests that s.a. is a normal metabolite of caproic acid (22). Rat liver homogenates show in metabolic experiments the same amount of acetic acid from s.a. as from caproic acid (23-27). Rabbit liver and kidney homogenates oxidize completely sorbic acid and other fatty acids to carbon dioxide and water (28). Extracts from pigs and beef heart, liver and kidneys, namely of beef liver, metabolize s.a. with the same speed as caproic acid (29) and mitochondria from carp liver behave similarly (92).

The metabolism tests in vitro seem to indicate that the decomposition of s.a. is identical to the decomposition of caproic acid and other natural fatty acids. Studies in vivo gave the same results. After feeding s.a. to fasted rats the same decomposition products were found in the urine as when caproic or other fatty acids were given (30). Metabolism of s.a. liberates 6.6 kcal (30, 117) from which about 50% are utilized in the biological test (117). (The energy value of 18 cal/g given in (31) is based on an error).

Sorbic acid undergoes β-oxidation in the human and animal body, i.e., following the decomposition mechanism given: then it is linked to coenzyme A. As this occurs quantitatively it was proposed for the determination of the coenzyme A (32, 38, 71).

Sorbinsäure		**Capronsäure**
		$CH_3-CH_2-CH_2-CH_2-CH_2-COOH$
		Dehydrierung durch Acyldehydrogenase $-2H$
$CH_3-CH=CH-CH=CH-COOH$	***α,β-ungesättigte Säure***	$CH_3-CH_2-CH_2-CH=CH-COOH$
$+HOH$ Hydratation durch Enoylhydratase		Hydratation durch Enoylhydratase $+HOH$
$CH_3-CH=CH-CHOH-CH_2-COOH$	***β-Hydroxysäure***	$CH_3-CH_2-CH_2-CHOH-CH_2-COOH$
$-2H$ Dehydrierung durch β-Ketohydrase		Dehydrierung durch β-Ketohydrase $-2H$
$CH_3-CH=CH-CO-CH_2-COOH$	***β-Ketosäure***	$CH_3-CH_2-CH_2-CO-CH_2-COOH$
$+2O_2$ Oxydation durch β-Ketothiolase		Oxydation durch β-Ketothiolase $+2O_2$
$CH_3-CH=CH-COOH+2CO_2+H_2O$	**um 2 C-Atome ärmere Fettsäure und Oxydationsprodukte**	$CH_3-CH_2-CH_2-COOH+2CO_2+H_2O$
		Dehydrierung durch Acyldehydrogenase $-2H$
$CH_3-CH=CH-COOH$		$CH_3-CH=CH-COOH$
	$+HOH$	Hydratation durch Enoylhydratase
	$CH_3-CHOH-CH_2-COOH$	***β-Oxysäure***
$-2H$		Dehydrierung durch β-Ketohydrase
	$CH_3-CO-CH_2-COOH$	***β-Ketosäure***
$+2O_2$		Oxydation durch β-Ketothiolase
	$CH_3-COOH+2CO_2+H_2O$	

Sorboyl-coenzyme A undergoes hydration by enoyl-hydratase (crotonase) to β-hydroxy acid. The latter is dehydrogenated by β-ketohydrase (β-hydroxyacylhydrogenase) to the β-ketoacid which is hydrolyzed by β-ketothiolase (β-ketoacyl thiolase). Reaction products are a fatty acid containing 2 carbon atoms less, CO_2 and H_2O. The newly formed fatty acid undergoes the same degradation so that carbon dioxide and water are formed as end products compared to the decomposition of caproic acid. The first reaction step is ommitted namely the α,β-dehydrogenation since s.a. already has a double bond in theα,β-position. The scheme shows the pathway of s.a. and caproic acid decomposition (15, 33, 34, 53, 63).

When extremely high doses are used we can observe in analogy to conventional nutritional acids - a small ω-oxidation together with the β-oxidation given in detail. When 10 g of s.a. was given daily to rabbits trans-trans-muconic acid in 0.1 - 0.2% amounts calculated to s.a. was present in the urine (14). ε-Hydroxysorbic acid was detected as intermediary products (93). Ester and nitrogen-containing derivatives of sorbic acid namely up to 44% of methylamide of s.a. are converted during passage through the rabbit into the corresponding muconic acid derivative as the amide group suppresses β-oxidation. While s.a. and its lower aliphatic alkyl esters show no pharmocological activity a dose of 1 g of sorbic acid amide and amide derivatives per kg of body weight and day in the rabbit causes transient paralysis (14). When sorbic acid amide is fed, up to 14% of the acid is eliminated in the form of muconamide in the urine as a result of ω-oxidation (35). The degradation mechanism of sorboylhydroxamic acid whose LD_{50} in intraperitoneal application is only 0.7 g/kg (66) is not sufficient known.

Tests with radioactively labeled s.a. further show that it is decomposed and utilized metabolically similarly to other nutritional fatty acids. When 61-1213 mg of 1-^{14}C-sorbic acid were given per kg of body wt. to rats then independently of the dose 85% was eliminated as $^{14}CO_2$. The rate of oxidation is dose-dependent and led to a half-time value of 40 to 110 min. S.a. is resorbed practically quantitatively from the intestine. Within 6-8 hours after administration the following distribution of activity was obtained:

Exhaled air	85% as $^{14}CO_2$
Feces	0.4%
Urine	2% as NH_2-^{14}CO-NH_2
Internal Organs	3%
Muscles	3%
Skeleton	6.6%

Sorbic acid does not form glycogen but is in a small part used for new synthesis of specific fats since some activity was detected in the subcutaneous fats. The scope and rate of oxidation of labeled s.a. and labeled caproic acid are identical, both fatty acids have a similar metabolic behavior (36, 65). According to data of other authors in the mouse after feeding 40-3000 mg/kg of body wt. of 1-^{14}C-s.a. was found the following distribution of activity independent of the dose (37):

Exhaled air	81 ± 10% as $^{14}CO_2$
Urine	4%
Feces	1%

Contrary to previous studies in (15, 36) the first 24 hours after feeding 0.7% of s.a. itself and 0.4% as trans-trans-muconic acid were seen in the urine. This test confirmed, however, the quantitative utilization of s.a. in the body (37).

f. <u>Effect on the skin:</u> Since s.a. is used for the preservation of cosmetic preparations and other external medications its skin tolerance was tested. Data in the literature are contradictory; there are indications that s.a. in some, obviously very sensitive people causes skin irritation, but experimental data shows in general that s.a. can be used without dermatological hesitation.

S.a. irritates the mucous membrane of the eye when introduced in the eye as powder or solution (40, 41).

Rags soaked with a 15% s.a. solution cause after 1 hour contact with the skin transient reddening in some people, but disappeared without after effect. Particularly sensitive people react already to a 0.01-0.02% aqueous solution or a 0.025 to 0.05% water-in-oil emulsion of s.a. (42). In guinea pigs s.a. in the rag test did not cause allergy (43) and even a 50% suspension in silicon oil had no acanthotic effect (44). Longlasting painting of the skin with solutions of the dimeric sorbic acid monoglyceride did not cause an abnormal condition (69).

In systematic studies with a large number of people 49 out of 50 tolerated the application of water-containing emulsion ointments containing up to 4% of s.a. Only one person reacted after the third application with some phenomena of irritation (45). Water-in-oil, oil-in-water emulsions, waterfree paraffin oil-ointments and traganth-glycerol-creams each with 10% of s.a. showed in repeated applications signs of irritation in 10 out of 1489 people tested. These could be traced in 5 people to the irritation effect caused by the high acid content. With water-in-oil emulsions the number of irritations found was only very slightly higher than in other preparations-containing s.a. However, these results cannot be considered as significant.5% solutions of s.a. in 86% alcohol or a 5% s.a.-jelly caused in one long-lasting test stretched over more than 26 days transient signs of irritation in only 7 out of 47 patients (46). These results were confirmed. The particular eczematic skin reaction observed in the rag test with 2%-solutions of s.a. and potassium sorbate and with their preparations result from contact dermatitis. 5 out of 736 persons with eczema and 2 out of 38 people without eczema reacted to an ointment with 2.5% s.a. by the appearance of transient reddening of the skin, occasionally with blister formation. Guinea pigs treated repeatedly with 20% potassium sorbate ointments show formation of erythema (52).

A eucerine ointment containing 2.5% of s.a. therefore a dose 10-times higher than usual, caused a reaction in 2 different observation periods in 1.8 resp. 3.2% of the eczema-suffering people tested (94).

Rubbing s.a. or -sorbate-containing 5% lanoline-vaseline ointments for 21 days on the shaved skin did not cause any significant changes in the gain of wt. of rats; regretfully when copper sorbate was used diarrhea occured which can be explained by the resorption of copper (84). Signs of hypersensitivity of the skin appeared in guinea pigs after 10 days of a daily injection with 0.1 ml of a 0.5%- sol. of sodium sorbate in 10% horse serum; antibodies were formed in the serum (76).

The previous studies and results lead to the conclusion that the threshold of irritation of s.a. is above 1%, therefore far above the normal concentration used of 0.1 to 0.3%. Sorbic acid can indeed also cause local irritations of short duration with lower concentrations (60, 70, 113, 116, 120). But the probability of this is relatively low; s.a. therefore does not represent any higher risk than any other agent used for the preservation of ointments, rather the contrary is true.

g. General evaluation: S.a. should be considered as an unobjectionable compound on the basis of numerous studies made, even according to modern ideas. Not only is its acute toxicity very favorable; also average-and long-lasting tests showed its complete non-hazardous nature. The fact that s.a. is decomposed in a manner similar to other fatty acids justified its special position among the known and presently used preservation agents.

It is obvious that there is barely a preservation compound whose behavior in the body was studied in such a detail and is known as s.a; contrary to other preservation agents it does not require in the body a "detoxication mechanism" (80, 95, 96, 121).

S. a. is listed on the international level by the commissions of experts, unanimously as a harmless preservation compound. The "International Union against Cancer" U. I. C. C. lists s. a. on the A list among compounds which should be considered on the basis of animal tests as probably not toxic and non-carcinogenic and, therefore, tentatively approved for human consumption (57). The "Commission of experts for food additives of the West-European Union" (WEU) classified s. a. in list I together with kitchen salt, acetic acid, citric acid, glycerol, i. e., among compounds considered harmless for human health (48).

At the 4th conference of the continuing European study committee for the protection of the population from chronically-toxic environmental damage (EUROTOX) a proposed list of acceptable food preservation compounds was presented. S. a. is included together with sugar, salt, acetic acid in the group of compounds which can be considered as non-dangerous without special limitations of amount (49).

The combined committee of WHO/FAO (Joint Expert Committee on Food Additives) of the United Nations (UNO) considers sorbic acid as the preservation material most highly tolerated in unlimited use (50, 79). The "acceptable daily intake" i. e., the amount added per day which can be consumed without danger was set according to WHO/FAO for s. a. as 12. 5 mg. /kg. body wt. (unconditional) or 25 mg. /kg. of body wt. (conditional). The lower value is basically valid, the higher for special cases. Both values have a high security range. The level given for daily human consumption of 1 to 2 g. is in practice never reached (125).

Comparative values for some other preservation compounds are given in the following table (50, 79).

Preservative	Acceptable daily intake unconditional	conditional
Sorbic acid	0 - 12.5	12.5-25
Propionate	0 - 10	10-20
Benzoic acid	0 - 5	5 - 10
pHydroxy benzoate	0 - 2	2 - 7
Formic acid	-	0 - 5
Sulfurous acid	0 - 3.5	0.35 - 1.5
Biphenyl	0 - 0.05	0.05 - 0.25

Sorbic acid answers all points of the requirements of the committee of the "Commission Internationale des Industrie Agricales" (CIIA) and of the "Bureau International Permanent de Chimie Analytique" (BIPCA (48, 51). In direction for the levels of the European Economic Community for equalization of the prescriptions of the member states for presevation agents used in food stuffs" s.a. and its sodium, potassium and calcium compounds are listed in first place in position E200 to E203 (39). On the basis of recommendations of national committees s.a. is presently accepted in practically all countires of the earth for the preservation of food stuffs (127-1211).

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